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Eric E. Sabelman, University of California at San Francisco, San Francisco, California
Peter V. Chetirkin
Robin M. Howard, Ames Research Center, Moffett Field, California

National Aeronautics and
Space Administration

Ames Research Center
Moffett Field, California 94035

INTRODUCTION

On September 25, 1979, the Soviet Cosmos 1129 biosatellite carried five female and two male rats into orbit. It was intended that the animals would mate in space and that the females would spend the first 1 to 16 days of gestation in near-weightlessness before reentry and parturition at normal gravity (35). This joint U.S./USSR experiment was the first attempt to study the effects of spaceflight on mammalian reproduction. Previous experiments on fish egg fertilization (7) suggested detrimental consequences, but experiments on later embryogenesis and development of frogs (36), fish (20), and fruit flies (24) showed no deleterious effects. On Cosmos 1129, neither flight females nor synchronous ground-control rats exposed to simulated launch and reentry successfully gave birth. This paper reports the results of two simulation experiments in which the possible causes of the failure of the Cosmos 1129 animals to carry normal pregnancies to term were explored. One experiment examined effects of reentry stresses on females at known gestation stages, and the other was a simulation of the complete flight profile.

Soviet investigators have hypothesized that stresses of reentry caused total fetal resorption in Cosmos 1129 rats. The resorption hypothesis appears reasonable in view of published experiments on the effects of multiple restraint stresses on rats (1, 2, 5, 11). Short-term physiological and psychological stresses caused postnatal developmental retardation, low birth weight, and increased fetal mortality or failure of uterine implantation. Complete resorption of entire litters

was a relatively uncommon effect. Time of application of stress during gestation was a critical factor (28); stress during a sensitive "window" produces a characteristic pattern of defects, although similar stresses at other times may have no effect (4). Resorption could occur by death of fetuses during or immediately after application of stress, or could be delayed, for example, until fetal growth overloaded impaired placental blood vessels (10).

The null hypothesis in reentry simulation was that stresses and control animals impregnated simultaneously would give birth at the same time to litters of the same size. The complete flight simulation tested the additional assumptions that: (1) launch stresses had no effect on time of impregnation relative to cage and diet controls, and (2) caging, diet, and other factors had no effect relative to vivarium control animals or previous mating of the same animals. Animals were sacrificed to examine uterine contents before resorption could proceed to the point that no fetal remains were detectable. The magnitude of the stress perceived by the test animals was assessed by behavior and appearance at the conclusion of each simulated flight parameter and by the masses of the adrenal glands of sacrificed animals (3, 9).

METHODS AND MATERIALS

Reentry simulation: Timed-pregnant outbred Wistar rats, initial mass of 210-230 g, were obtained from Simonsen Laboratories. The supplier verified vaginal plugs on the day following mating. On receipt, animals were 12, 10, 8, 6, 4, 2 d and 12 hr pregnant. Each animal was identified

based on gestation time at receipt (e.g., 12D = 12 days; 12H = 12 hours). Animals were weighed to within ±0.5 g (Figs. 1-3) and were given Simonsen G4.5 breeding chow and water ad lib.

On the day of the test, animals were assigned at random to one of three groups: two to be stressed (groups TA-1 and TA-2) and one control (TA-3). A blood sample was drawn by clipping the tip of the tail of group TA-3 rats; plasma specimens were intended for glucocorticoid and progesterone assays, which are not included in this report.

The individually caged animals were moved from the vivarium to the test building. The control animals (TA-3) were transferred to a single test chamber as a group and placed in a quiet area. The two test groups were in turn placed in separate test chambers, exposed to simulated reentry, examined for immediate behavioral and appearance responses, and returned to individual cages. On conclusion of testing, a second blood sample was drawn from the TA-3 group. All animals were returned to the animal holding facility and left there undisturbed for 2 d.

On day 3 after reentry simulation, the TA-1 group was individually weighed, decapitated, and exsanguinated. They then were laparotomized and a photograph was taken of the internal organs with uterus displayed. Each uterus was dissected from the vagina, mesentery, and accompanying adipose tissue; ovaries were retained with the uterus. The uterus was placed in a Petri dish containing normal saline and was photographed under transmitted oblique illumination. Prior to photography, uteri over 12 d gestation were opened to expose amniotic sacs. The adrenal glands were excised, cleaned of adherent fat, and weighed to the nearest 0.1 mg.

The TA-2 and TA-3 groups were inspected and weighed daily. If a female in the TA-2 group did not show any weight gain for 3-4 d, she and the corresponding TA-3 female were sacrificed by the above procedure. Otherwise, as soon as possible after birth was complete, offspring were counted and their condition and total mass were assessed. Litter size was reduced at random to 8. The litter was weighed on alternate days until mean mass of the pups was 30 g.

Complete flight simulation: Animals were from second and third generations of the Ames Research Center (ARC) Czech Wistar breeding colony originally obtained from the Slovakian Academy of Sciences, Bratislava. They were 6.5 to 7.5 months old and were proven breeders, each having had one litter of seven or more pups 2.5 months previously. Females were selected at random from a pool of 20; littermates were kept under vivarium conditions. Females were housed 4-6 per cage until 6 days prior to simulated launch; then they were placed in individual cages, identified by tail-mark code, acclimated to group placement in test chambers for 1 hr daily, and fed 45 g of Soviet paste diet (22) once daily.

Five females and two males were placed in each test chamber on the day of simulated launch (L+0) and transported to the simulated facility. Groups TS-1 and TS-2 were successively exposed to vibration and acceleration, and group TS-3 was placed in a separate quiet room.

Males were selected from littermates caged together; inadvertently, one set of males (TS-2) had not previously been acclimated to common caging; consequently dominance/submission interactions occurred during the first 3 d of the simulated mission. Males were also weighed, isolated, and fed the Soviet paste diet 4 d before start of the test.

Upon completion of launch simulation, the chambers were moved to an isolation room for 18.5 d. During this period, paste diet (55 g per rat per day) was given in eight feeding cups per chamber twice daily at approximately 0800 and 1600 hr. Uneaten food was weighed and included in the allocation at the next scheduled feeding. Water was available ad lib at six locations in each chamber. Temperature varied between 70° and 74°F (21°-23°C). Relative humidity was 50-64%. Illumination was 16 fc on a 12-hr-on, 12-hr-off cycle. Waste trays filled with shavings or absorbent clay were changed every 1-2 d. Groups TS-1 and TS-3 were undisturbed except for changing food, water, and waste trays. Females of group TS-2 were removed at 2-3 d intervals for weighing.

On day L+2, partitions separating males from females were removed, thus permitting the rats to mate. On three occasions, beginning on day L+11, vaginal smears were taken from TS-2 females to determine estrous phase or presence of sperm (22).

Reentry was simulated by moving the chambers back to the test facility, exposing groups TS-1 and TS-2 to acceleration and shock, examining for immediate responses, and then returning the animals to the isolation room. Females were weighed, placed in individual cages, and given 60 g/d of paste diet once daily for 14 d or until birth occurred; they were fed standard rat chow thereafter. On the day of reentry the female having greatest relative weight gain in each group was sacrificed by decapitation. Five and seven days after reentry one additional female from each group was sacrificed; the remaining females were permitted to carry pregnancies to term and were sacrificed after weaning of litters.

Animals were dissected as in the reentry-only experiment with the addition of dissection and weighing of fat deposits from the abdominal cavity. Six littermate females were sacrificed for comparison of abdominal fat (Groups CTR-A and CTR-B).

Test chamber and simulation environment: Throughout the flight-simulation experiment the three groups of animals were housed in three experimental chambers (Fig. 4a) similar to the one used in the Soviet Cosmos 1129 ontogeny experiment (Fig. 4b). Simplified chambers, lacking feeding cups, water bottles, and waste trays, were used in the reentry-only experiment. The chambers were constructed of aluminum with a perforated acrylic top and a stainless steel mesh floor. The dimensions (length × width × height in centimeters) of the chambers used in this experiment and in the Soviet study were as follows:

1. Soviet study

   Female section: 48.0 × 20.0 × 16.0

   Male section: 20.0 × 17.0 × 16.0

2. Present study:

   Female section: 48.3 × 22.8 × 15.8

   Male section: 22.8 × 22.8 × 15.8

An 11-cm-diameter fan was attached to the top of each chamber to increase airflow. Soviet-supplied launch and reentry noise, vibration, acceleration, and shock data from previous Cosmos missions were used for simulation parameters.

Random Motion Vibration. Each chamber was vibrated parallel to the vertical axis for 10 min. During the vibration test the noise level was monitored at 85 dB. The vibration amplitude, s(f), was constant as linear within the following frequency ranges:

Frequency, f	s(f)
20-100 Hz	0.014-0.028 g^2/Hz
100-400 Hz	0.028 g^2/Hz
400-2000 Hz	0.028-0.02 g^2/Hz

Acceleration. Following vibration and visual inspection, test groups were subjected to 4.0 ± 0.2 g's in the vertical axis for 10 min, of which the first and last 90 sec were linear ramp functions. Mounting axes permitted each unit to swing freely during acceleration. For simulated reentry, the test groups were subjected to 6.0 ± 0.2 g's radial acceleration for 5 min, of which the first minute was at a linear rate to 6 g; the rate was decreased linearly to stationary during the last minute.

Impact Shock. Following visual examination each chamber was secured on the impact apparatus platform and subjected to a half sinusoidal 50 ± 3 g shock for 1 msec parallel to the vertical axis.

RESULTS

Moderate hypoactivity was the only immediately observable behavior attributable to stress response. Control groups also were relatively inactive after initial exploration of the test chamber. Total reproductive success for the reentry simulation is summarized in Table I and for the full flight simulation in Table II.

Reentry simulation: Normal pregnant uteri, as determined by gross visual examination, were present in all TA-1 animals except 8D. Examination of the 8D uterus revealed no vascular enlargement, implantation sites, or resorbed fetal remains. Mass gain prior to sacrifice was normal (Fig. 1), except for 8D and 12D; the latter was smaller initially and had the smallest litter size. Animal TA-1:10D had one advanced resorbing fetus.

Five TA-2 animals carried pregnancies to term (Fig. 2). TA-2:6D gained 30 g more than any other, and gave birth to 13 live pups and 1 stillborn. The weight of TA-2:10D was consistently at least 20 g less than that of the other animals at corresponding stages of pregnancy; 10D had a litter of only 8 pups. Several of the successful pregnancies had transient mass losses or plateaus 4-6 d after the reentry simulation. TA-2:8D had only a small gain between receipt and testing, and less gain following the test than other animals. This animal and the corresponding TA-3 animal were killed 4 d after the simulation, on the presumption of resorption; however, there were no indications of pregnancy. TA-2:2D had normal gain for 4 d after simulation, but then began losing mass. At 16 d gestation, this animal and the corresponding TA-3 animal were killed. No placentation scars or fetal remains were evident in TA-2:2D.

Of the two TA-3 animals sacrificed on the 16th day of gestation, one (TA-3:8D) had 11 viable fetuses and one resorption (Fig. 5). State of resorption was such that fetal death most probably occurred 1 or 2 d after the test. The TA-3:2D animal also had 11 fetuses. All were underdeveloped for 16 d gestation. Amniotic blood vessels were thickened, amniotic fluid was cloudy, and there was little limb modeling. The five

remaining TA-3 animals carried pregnancies to term (Fig. 3). Mean litter size was 11 ± 0.58, slightly but not significantly larger than live births in the TA-2 group (10 ± 1.87 SD). Several animals had decreases in rate of weight gain 4-6 d following the test (TA-3:12H, 2D, 6D). Only one (4D) plateaued, and none lost weight.

Full flight simulation — animal mass: All females gained mass during the flight period (66.0 ± 25.9 g). Excluding one possibly underfed animal, which gained only 9 g, the mean gain was 70.1 ± 21.3 g. Vivarium controls gained 13.2 ± 10.9 g during the same period. Group TS-1 (Fig. 6) included the animals gaining the least.

Group TS-2 (Fig. 7) included one exceptionally large mass gain, with the others tending to be more closely clustered than at the start of the test. Postflight, all but one female showed consistent exponential gain indicative of pregnancy.

Group TS-3 (Fig. 8) included one animal larger than the others from the outset and also included the animal gaining most relative to its initial weight. The other three were within a 20-g range at both the start and finish of the flight period. One of these continued to gain while the other two plateaued.

As in the reentry simulation, there were disturbances in rate of mass gain in animals in each group in the 3-5 d period after reentry. In group TS-1, two animals (which later had normal parturition) showed transient losses, as did one animal sacrificed after a mass loss that began earlier. Similarly, one TS-2 animal lost mass and one peaked, followed by restoration of exponential gain and parturition. In

group TS-3, two animals had losses or plateaus beginning on the day of reentry. Other animals suffered only declines in rate of mass gain.

Full flight simulation – preterm reproductive success: Nine females were dissected prior to giving birth (Table II). Two sacrificed "flight" females had 12 viable fetuses with three resorptions. Gestation age, based on gross examination of the uterus, was 12 d. The control TS-3 female had only three placentations.

Selections for sacrifice 5 and 7 d after reentry were based on intermediate weight gain followed by plateau or loss. In both cases the TS-1 animals were not visibly pregnant upon gross dissection, one having fluid in the uterine lumen and the other kidney abnormalities. The TS-2 females were pregnant, but had low numbers of viable fetuses of gestation age 16-17 d (Fig. 9) and other well-developed but abnormal fetuses. The first TS-3 animal had only one normal and one small fetus; the second had nine fetuses of about 18 gestation days, again with one runt. Each animal had three resorbing fetuses.

Term pregnancies – reentry simulation: There was a tendency for control females to give birth earlier (by about 0.8 d). There was also a trend toward heavier mean pup mass in control compared with TA-2 group offspring, which continued through the first 10 postnatal days. Preimplantation pregnancies (12H, 4D) at the time of test were exceptions to this trend.

Aside from one observed stillbirth (TA-2:6D), there was only one abnormal pup in group TA-2 and one in TA-3. One small pup of TA-2:12D had facial edema and a hematoma; this was regarded as a result of birth trauma, rather than effects of reentry simulation.

Term pregnancies — full flight simulation: Two females in each group exhibiting exponential weight gain carried pregnancy to term. The two in group TS-1 had 13 and 8 offspring, born 17 and 20 d, respectively, following "reentry." The two in group TS-2 had 8 and 6 pups, born 13 and 16 d, respectively, after last mating opportunity. And the two females in group TS-3 had litters of 10 and 11, both born 11 d after reentry. The delay of parturition after reentry of combined TS-1 and TS-2 groups relative to TS-3 was significant at the $P < 0.1$ level (Student's t-test).

Stillbirths occurred in groups TS-2 and TS-3. Four pups in the TS-1 litter of 13 appeared to have edema or hematomas or both in the intrascapular brown fat pad. The TS-2 litter of six was destroyed by maternal cannibalism. Twenty-six female and 14 male offspring were weaned. Postnatal mass gain appeared to coincide with the curve followed by other Czech Wistars in the ARC colony (32).

Two TS-2 and two TS-3 females were dissected after their litters were weaned; one of these had died within 12 hr previously, because of the rupture of an aortic aneurysm. Only one was still lactating 24 d after parturition. About the same number of placental scars (implantation sites) on the uterus were detectable as the sum of the two litter sizes borne by the female.

When the numbers of live births and viable fetuses were combined (Table III), there were no statistically significant differences between groups. When compared with mean number of live births in the previous litters of the same animals, that of each TS group was significantly lower, with a combined mean of 6.9 births/litter compared with 10.3 previously ($P < 0.05$). If the two TS-1 females without new placentations

are excluded as possibly unimpregnated, the mean viable litter size was 8.08 ± 3.52 ($P < 0.1$). Unsuccessful fetuses (resorptions and stillbirths) were elevated in all three groups compared with previous litters (1.3 vs 0.2 per litter). Since not all resorptions and stillbirths were detected, no statistical comparison is possible.

Adrenal mass – reentry simulation: The mass of both adrenal glands of each animal fell within the range of 40 to 70 mg (Table IV). These masses are best expressed as a ratio of adrenal gland mass to body weight (9); when not measured, initial masses of pregnant animals were estimated by extrapolation of the gain curve to day 0. Adrenal:body mass ratios were in the range of 1.5×10^{-4} to 3.0×10^{-4}, with slight clustering around 1.9×10^{-4} and 2.9×10^{-4}. Animals with ratios near 2.9×10^{-4} had normal pregnancies. The data of Geller et al. (9) for both group-housed and chronically isolated animals are near the low end of the range of the present study (Table IV). Other data (12) for grouped females are in the middle of the range; isolated females have higher adrenal mass ratios. Rigorous comparison with the present study is difficult because of differences in animal age and sex.

Adrenal mass – full flight simulation: There was no significant difference between any of the test groups, but the mean of combined adrenal weights was greater than that of vivarium controls ($P < 0.1$). Absolute adrenal masses of females caged with juvenile litters at the time of sacrifice were larger than usual. They also differed in appearance, being gray and granular rather than pink and homogeneous, which is felt to be a physiological response to the stresses of motherhood (25).

Pregnant females had slightly higher adrenal mass ratios (2.51×10^{-4}) than three nonpregnant females on paste diet (2.24×10^{-4}).

Abdominal fat: Dissectible fresh fat mass after full flight simulation was compared as a ratio of fat mass to body mass at the time of sacrifice (Table V). Females with unweaned litters that had been fed standard rat chow after discontinuing the paste diet were excluded. There were no significant differences between groups, but the entire population had a mean abdominal fat that was 10.4 ± 1.3% of body weight; this was a highly significant difference ($P < 0.001$) from vivarium controls (5.7% fat). There was no significant difference between females dissected during pregnancy and nonpregnant animals. Females with unweaned litters had fat-to-body ratios below the mean for vivarium controls; on examination, these animals had pink vascular fat of less viscous consistency, indicating fat resorption.

DISCUSSION AND CONCLUSIONS

Reentry simulation: Transient decline or plateau in mass gain following reentry simulation may be an indication of resorption of some but not all fetuses. Since control animals also showed decreased rate of gain at this time, handling or isolation stresses may have been sufficient to cause this effect. Late pregnancies (10D, 12D) were not subject to such fluctuation in weight gain, indicating relative insensitivity at the time of the test (14 and 16 d, respectively).

Recent resorption of early pregnancies could not be assessed from the external appearances of the uteri, although by the time of dissection

(8-10 d gestation) swellings at implantation sites were obvious. Vascular enlargement in early pregnancies had not progressed to the point that placentation scars would be evident long after resorption or normal parturition. Hence, animals termed "nonpregnant" (TA-1:8D, TA-2:2D, and TA-2:8D) may have been early postimplantation resorptions. Isolated resorptions (TA-1:10D, TA-3:8D) caused no difficulty in interpretation. The one animal with develcpmental retardation of the entire fetal litter (TA-3:2D) may have been in the early stages of resorption, but gave no other indication of abnormality, such as decreased mass gain.

Full flight simulation: None of the factors of the simulated Cosmos mission – which included (1) Soviet paste diet, (2) launch vibration, noise, and acceleration, (3) group housing in a confined volume, (4) competition for limited food, (5) restricted illumination and airflow, (6) reentry shock and acceleration, and (7) postflight handling and isolation – were able to prevent establishment and maintenance of pregnancy to term in all animals. There were no significant differences in the number of live fetuses and births or in adrenal mass ratios between the control group and the two groups exposed to launch and reentry stresses.

The 4.5 d mean delay of parturition of the TS-1 and TS-2 groups was the only statistically identifiable difference from the control group TS-3. This suggests that some factor may inhibit or resynchronize estrous cycles or cause temporary male reproductive dysfunction. However, a test of male reproductive capability following launch stresses yielded a normal number of successful litters from matings within the 4-d period beginning 2 d after "launch" (E. Megory, unpublished data).

Even controls (TS-3) did not mate on first opportunity after the partition separating males and females was opened. If the mean gestation period was 22.5 d (17), then mating in TS-3 occurred 5 d (L+7) after the partition was opened. Only one TS-1 mating preceded the mean TS-3 mating time (see Table I). Mating of one TS-2 female at L+11 d was confirmed by vaginal smear. All other TS-2 females estimated to be pregnant by findings of leucocytes in vaginal smears were confirmed by dissection or parturition. The longest delay following first mating opportunity was in a TS-1 animal in which mating took place 2 or 3 d before reentry. This animal gained very little weight during the simulated mission and could have been nutritionally anestrous for part of the incubation period. In a separate experiment (19), one male and three female Czech Wistar rats were housed together and fed the Soviet paste diet under conditions similar to those of group TS-3 in the present study. Litters of normal size were born from two matings on day 6 and one mating on day 17 after introduction of the males. Hence, a factor unrelated to simulated launch stresses appears to be the cause of at least the initial 5-d delay in impregnation in the present study as well. The assumption that fertile mating would occur as soon after opening of the partition as permitted by the estrous cycles of the females (within 4 d) therefore appears to be invalid.

The fact that mating of group-housed rats was delayed, whereas individually housed vivarium-caged males mated normally following launch stresses, may have a bearing on results of the Cosmos 1129 experiment. If other factors, such as locomotion in weightlessness, caused additional delay in mating, then the effective period available for mating would

have been still further reduced. Instead of the nominal mating opportunity of 16.5 d, or the 11 or 12 d evidenced by timing of TS-3 births, the mating period may have been only the last 4 or 5 d of the mission. The reproduction success of the TS-1 group, in which three of five females had no placentations at the time of reentry, as detected by dissection, or had a 20-d delay in parturition following reentry may closely correspond to that of the Cosmos 1129 flight animals.

Based on gross examination of fetuses, there were several causes for fetal mortality: some females had low numbers of detectable implantations, some fetuses were resorbed early relative to survivors, and others had anomalies in fetal anatomy (a prolonged abdominal hernia) or in placental function (detachment or discoloration). Stillbirths appeared to be anatomically normal. A sex-linked trait may have been partially responsible for decreased litter sizes, since females outnumbered males in all weaned litters. If the published (21) incidence of male births of 48.44% is applicable to Czech Wistars, then the probability of the observed incidence of 14 males in 40 live births is only 3.01%. However, since the gender of stillbirths and culled offspring was not recorded, there is no way to ascertain that weaned litters constitute a valid sample. The age of females may have been a factor in fetal mortality, since there is evidence that stress is more growth-inhibitory for progeny of older dams than of young females (8), although "older" animals in that report exceeded the age of Cosmos simulation animals by 4 to 6 months.

Abdominal fat, measured as ratio of fresh dissected fat mass to live body mass was significantly elevated in all three groups that had been fed paste diet, compared with littermate controls ($P < 0.001$). Similar

results were found in an earlier study with three rats fed a simulated Soviet paste diet (32). It has long been known that high-fat diets cause more fat buildup than equicaloric high-carbohydrate diets (30). Obesity-inducing diets are typically 60% (30) to 64% (33) fat, compared with 80% carbohydrate in standard diets.

Literature values of fat-to-body weight ratio vary depending on method of fat extraction. In the most applicable study available (30), the total body fat of female rats was divided into six depots. Genital, peritoneal, mesenteric, and omental depots contained 35-45% of dissectible fat, as determined by ether extraction. Nonextracted residue of 6.3% must be added to approximate fresh wet weight. Extracted fat of high-fat-fed females was 5.9 ± 1.5% of body mass, compared with 3.9 ± 1.2% in starch-fed rats. In a more recent study (33), in which fat was extracted from the homogenized whole body, high-fat-diet (180-290-g) female rats had 12.0% of the body mass in abdominal fat (assuming 35% of total fat in this depot), compared with 5.4% for grain-fed animals.

In the present study, the ratio of percent abdominal fat of paste-diet-fed females to chow-fed females was 1.82. Literature values for percent abdominal fat in 60%-fat-fed relative to carbohydrate-fed female rats range from 1.50 (30) to 2.23 (33). However, the Cosmos diet solids are stated to include only 11% fat (22), plus 60% carbohydrate. Thus, female rats fed the Soviet paste diet appear to have deposited fat in a manner similar to that of high-fat-fed animals.

Obesity apparently does not interfere with the ability of adult rats to survive spaceflight but could inhibit reproduction by interaction with other factors. In rodents, excess fat in the diet may affect pregnancy

by interference with serotonin metabolism (18) and hence pituitary secretion of gonadotropic hormones.

In humans, the interaction of psychological stress and pituitary function leads to failure of ovulation (29), probably by production of excess prolactin (31). The equivalent problem of anestrous female rats is less well documented (15). Fetal mortality of stressed females is known to increase (14), nursing behavior may be abnormal (13), and hypothalamic dopamine is increased in female progeny (27). Vasoconstrictive effects of stress-induced corticosteroids may be a factor, since vaginal hemorrhage is associated with fetal mortality in stressed rats (14). Vascular effects secondary to stress have been shown to cause fetal anoxia in primates (23, 26); if prolonged, such anoxia would cause fetal death. Vasoconstrictive mechanisms would be most effective in increasing near-term fetal mortality and still births, because placental degeneration normally begins prior to birth in all females. Chronic noise stress is known to alter fetal (37) and postnatal (21) development in rats. Isolation following flight also may be a stress factor (9, 34, 37), although one not capable of causing fetal mortality in Soviet-paste-diet-fed Czech Wistar rats in the absence of other factors (19).

The reduction in the number of ovulatory females may become more extreme if some females are anestrous because of insufficient, rather than excess, food intake. If the partial pressure of oxygen became deficient in the sealed atmosphere of the biosatellite or synchronous simulator, or if those atmospheres contained toxins such as carbon monoxide (R. A. Tigranyan and H. G. Vetrova, 1980, unpublished report), the fetus might become anoxic, even in the absence of vasoconstriction.

Alterations in photoperiod due to light leakage from experiments on other light/dark cycles, sounds produced by other animals, and other forms of interexperiment interference may occur in biosatellites. The above factors should be excluded from future flight experiments by rigorous preflight simulation studies.

The present simulation studies and reports in the literature imply that successful pregnancies can be obtained in female rats undergoing simulated spaceflight; however, effects have been observed that could explain the failure of Cosmos 1129 females to become pregnant on the basis of reduced probability of conception and fetal viability. The Cosmos 1129 population was sufficiently small that each animal could have been affected by a different combination of factors reducing its fertile interval during the flight or damaging fetuses if impregnated.

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TABLE I. REPRODUCTIVE SUCCESS: REENTRY SIMULATION.

Group	Female identification code	Gestation day at test	Gestation day at birth or sacrifice	Viable fetuses	Resorbed fetuses	Live births
TA-1	12D	16	19	9	0	
	10D	14	17	11	1	
	8D	12	15	0	0	
	6D	10	13	12	0	
	4D	8	11	10	0	
	2D	6	9	12[a]	0	
	12H	5	7	12	0	
TA-2	12D	16	23			10
	10D	14	22			8
	8D	12	16	0	0	
	6D	10	23			13
	4D	8	23			9
	2D	6	16	0	0	
	12H	5	23			10
			N = 5 22.8 ±0.45			
TA-3	12D	16	22			10
(control)	10D	14	22			11
	8D	12	16	11	1	
	6D	10	21			11
	4D	8	22.5			12
	2D	6	16	11	0	
	12H	5	22.5			11
			N = 5 22.0 ±0.61 t = 1.0645			

[a]Three small.

TABLE II. REPRODUCTIVE SUCCESS: FULL FLIGHT SIMULATION.

Group	Female identification code	First litter		Days after reentry	Post-test litter				Old implantation sites
		Live	Stillborn		Live births	Still-births	Viable fetuses	Resorbed fetuses	
TS-1	AA5.2 (2)	15		20	8	0			
(not handled)	AE2.8.4 (3)	10		0			12	2	
	AE2.9.5 (5)	10		7			0	0	12
	AE2.9.6 (7)	10		5			0	0	9
	AA5.3 (17)	12		17	13	?			
TS-2	AE2.7.1 (4)	11		13	8	1			
(handled)	AA5.4 (9)	7		16	6	?			
	AE2.9.2 (13)	10	1	7			6	2	
	AA5.5 (15)	7		5			5	4	
	AD4.2.4 (19)	12		0			12	0	
TS-3	AA5.6 (6)	7		11	10	2			
(cage and	AC1.8.1 (21)	12	1	11	11	0			
diet control)	AE2.9.1 (23)	10		7			9	3	
	AC1.8.3 (24)	11		0			3	0	
	AE2.8.3 (27)	10	1	5			2	3	
CTR-A	AE2.9.3	0							0
(standard	AE2.9.4	2	?						9
diet)	AE2.8.1	0							0
CTR-B	AE2.8.2 (14)	6							9
(standard	AE4.2.1 (22)	11							14
diet)	AE2.9	8							7

TABLE III. FETAL AND NEONATAL VIABILITY.

Number in litter (mean ±SD)

Group	N	Live births/fetuses	Dead births/fetuses
Initial breeding	15	10.27 ±2.15	0.22 ±0.44
TS-1	5	6.60 ±6.31	
	4		0.50 ±1.00
TS-2	5	7.40 ±2.79	
	4		1.75 ±1.71
TS-3	5	6.80 ±4.02	1.60 ±1.52
TS-1 + TS-2	10	7.00 ±4.62	
	8		1.12 ±1.46
TS-1 + TS-2 + TS-3	15	6.93 ±4.28[a]	
	13		1.31 ±1.44
TS-1 + TS-2 + TS-3 (excluding 0 implants)	13	8.08 ±3.52[b]	

Significance vs initial breeding: [a] $P < 0.05$; [b] $P < 0.10$

Birthdate relative to simulated reentry

Group	N	Delay (days, mean ±SD)
TS-1	2	18.5 ±2.12
TS-2	2	14.5 ±2.12
TS-3	2	11.0 ±0
TS-1 + TS-2	4	16.5 ±2.89[b]

Significance vs TS-3: [b] $P < 0.10$

TABLE IV. ADRENAL MASS OF SACRIFICED ANIMALS.

Group	Number of animals, N	Mean body mass, g	Adrenal mass to body mass ratio × 10^4 (mean ±SD)
TA-1	7	222[a]	2.23 ±0.49
TA-2	2	260[b]	1.99 ±0.64
TA-1 + TA-2	9		2.18 ±0.50
TA-3	2	223[a]	2.80 ±0.30
TS-1	3[c]	300[d]	2.36 ±0.58
TS-2	5	308[d]	2.61 ±0.22
TS-3	4[e]	305[d]	2.44 ±0.25
CTR-A + CTR-B	6	302	2.23 ±0.17
Geller et al. (9) (♀ + ♂)			
isolated		164	2.13
group		174	1.93[g] P < 0.05
Sigg et al. (34) (♂)			
isolated		500	1.30
group		542	0.81[g] P < 0.01
Hatch et al. (12)	20	(f)	
isolated ♂			1.3
group ♂			1.1
isolated ♀			3.0
group ♀			2.4[g] P < 0.01

[a]Estimated pre-pregnancy, ±5 g.
[b]Actual mass.
[c]Two animals not sacrificed at time of compilation.
[d]From mean of two days pre-test.
[e]One animal cannibalized litter; not sacrificed.
[f]17 weeks old or less.
[g]Significant difference between isolated and group.

TABLE V. ABDOMINAL FAT AT SACRIFICE.

Group	Female identification code	Mass at sacrifice, g	Abdominal fat, g	Fat/post-test ratio
TS-1	AA5.2 (2)	299	(*a*)	
(not handled)	AE2.8.4 (3)	396	36.0	0.0909
	AE2.9.5 (5)	348	38.9	0.1118
	AE2.9.6 (7)	378	40.2	0.1063
	AA5.3 (17)	376	(*a*)	
			N = 3	0.1030 ±0.0108
TS-2	AE2.7.1 (4)	355	18.01	0.0507[b]
(handled)	AA5.4 (9)	353	33.10	0.0938
	AE2.9.2 (13)	386	46.20	0.1197
	AA5.5 (15)	371	28.70	0.0774
	AD4.2.4 (19)	427	51.00	0.1194
			N = 4	0.1026 ±0.0207
TS-3	AA5.6 (6)	335	–	(*b*)
(cage and diet)	AC1.8.1 (21)	399	20.4	0.0511[b]
	AE2.9.1 (23)	381	39.4	0.1034
	AC1.8.3 (24)	407	43.5	0.1069
	AE2.8.3 (27)	366	39.7	0.1085
			N = 3	0.1063 ±0.0026

TABLE V. Concluded.

Group	Female identification code	Mass at sacrifice, g	Abdominal fat, g	Fat/post-test ratio
CTR-A	AE2.9.3	308	22.4	0.0727
(standard	AE2.9.4	291	14.8	0.0509
diet)	AE2.8.1	300	15.6	0.0520
CTR-B	AE2.8.2 (14)	301	11.66	0.0387
(standard	AD4.2.1 (22)	324	22.6	0.0698
diet)	AE2.9	286	—	
			N = 5	0.0568 ±0.0142

[a]Not sacrificed at time of compilation.

[b]Fat resorbing; standard diet; not included.

FIGURE CAPTIONS

Fig. 1. Mass of pregnant females exposed to reentry as a function of gestation day: Group TA-1 (sacrificed 3 d after test).

Fig. 2. Mass of pregnant females exposed to reentry as a function of gestation day: Group TA-2 (sacrificed only if low mass gain).

Fig. 3. Mass of pregnant control females as a function of gestation day: Group TA-3 (sacrificed with corresponding TA-2 female).

Fig. 4. Rat isolation chambers for mating-embryology experiments: (a) U.S. chamber for simulation studies, mounted on vibration platform for launch simulation with 300-g rats; (b) USSR Cosmos 1129 flight backup chamber, with 150-g rats. Legend: M = male rat compartment; F = female rat compartment; P = partition with removable sliding door; W = feed and water dispensing apparatus (water bottles removed in U.S. chamber); A = activity monitor; V = ventilated cover.

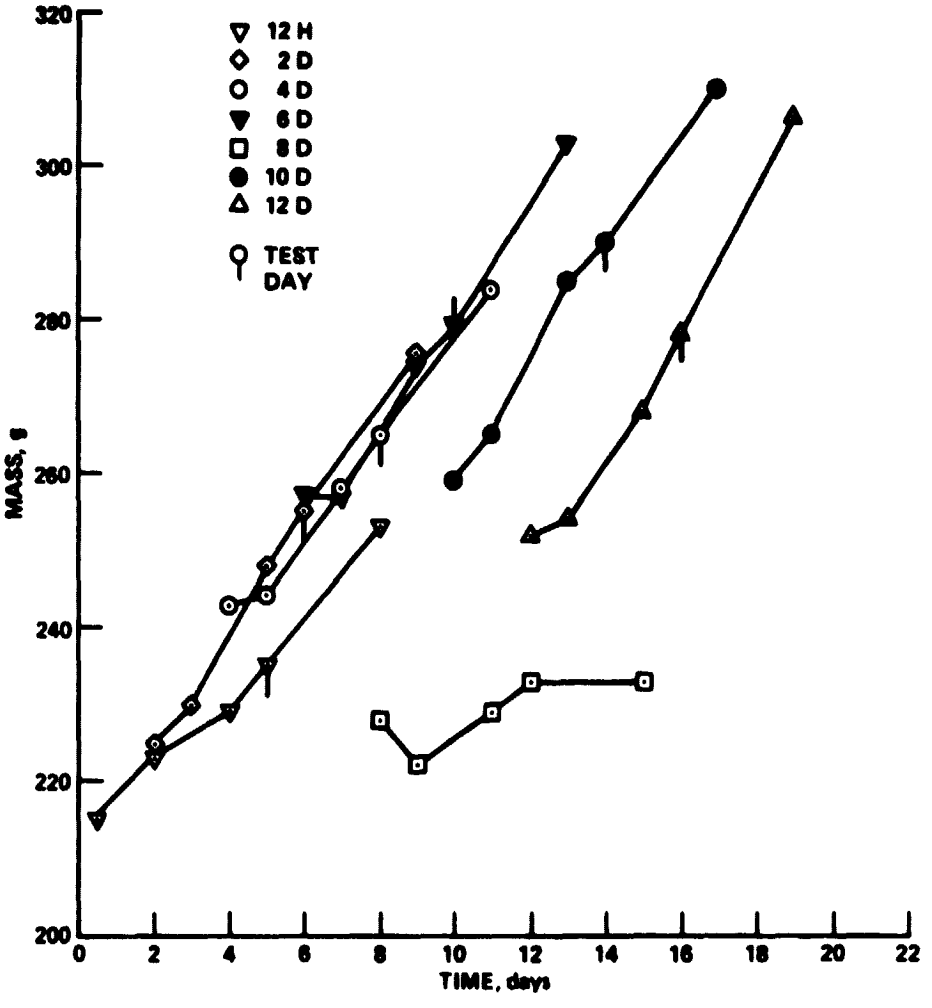
Fig. 5. Dissected pregnant control uterus, 16 d gestation (specimen TA-3 8D). Legend: F = viable fetus in amniotic sac (11 total); P = placenta; U = uterine wall (reflected); R = resorbing fetus; O = ovary; V = vaginal junction of uterine horns. (Scale (background): 1 cm.)

Fig. 6. Mass of females during full flight simulation: Group TS-1 (not handled during incubation).

Fig. 7. Mass of females during full flight simulation: Group TS-2 (handled for weighing and vaginal smear).

Fig. 8. Mass of females during full flight simulation: Group TS-3 (not handled during incubation).

Fig. 9. Pregnant uterus at laparotomy following simulated flight, approximately 16 d gestation (specimen TS-2 AA5.5-15, day L+5). Legend: R = right uterine horn (three fetuses); L = left uterine horn (six fetuses: two nearest vagina viable, two abnormal but living, two advanced resorption); V = vaginal junction of uterine horns; F = abdominal fat. (Scale bar: 1 cm.)



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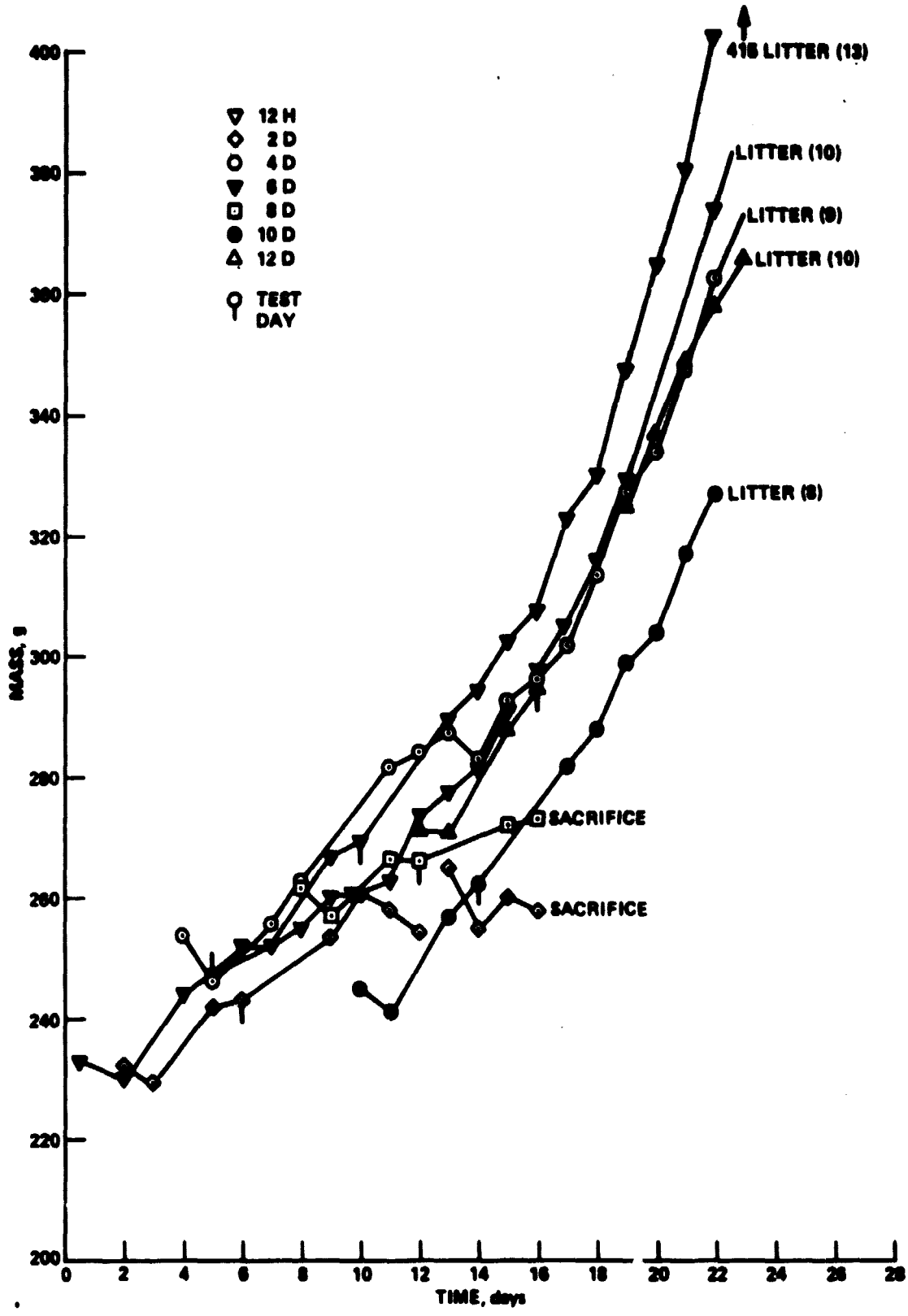


Figure 2.- Mass of pregnant females exposed to reentry as a function of gestation day: Group TA-2 (sacrificed only if low mass gain).

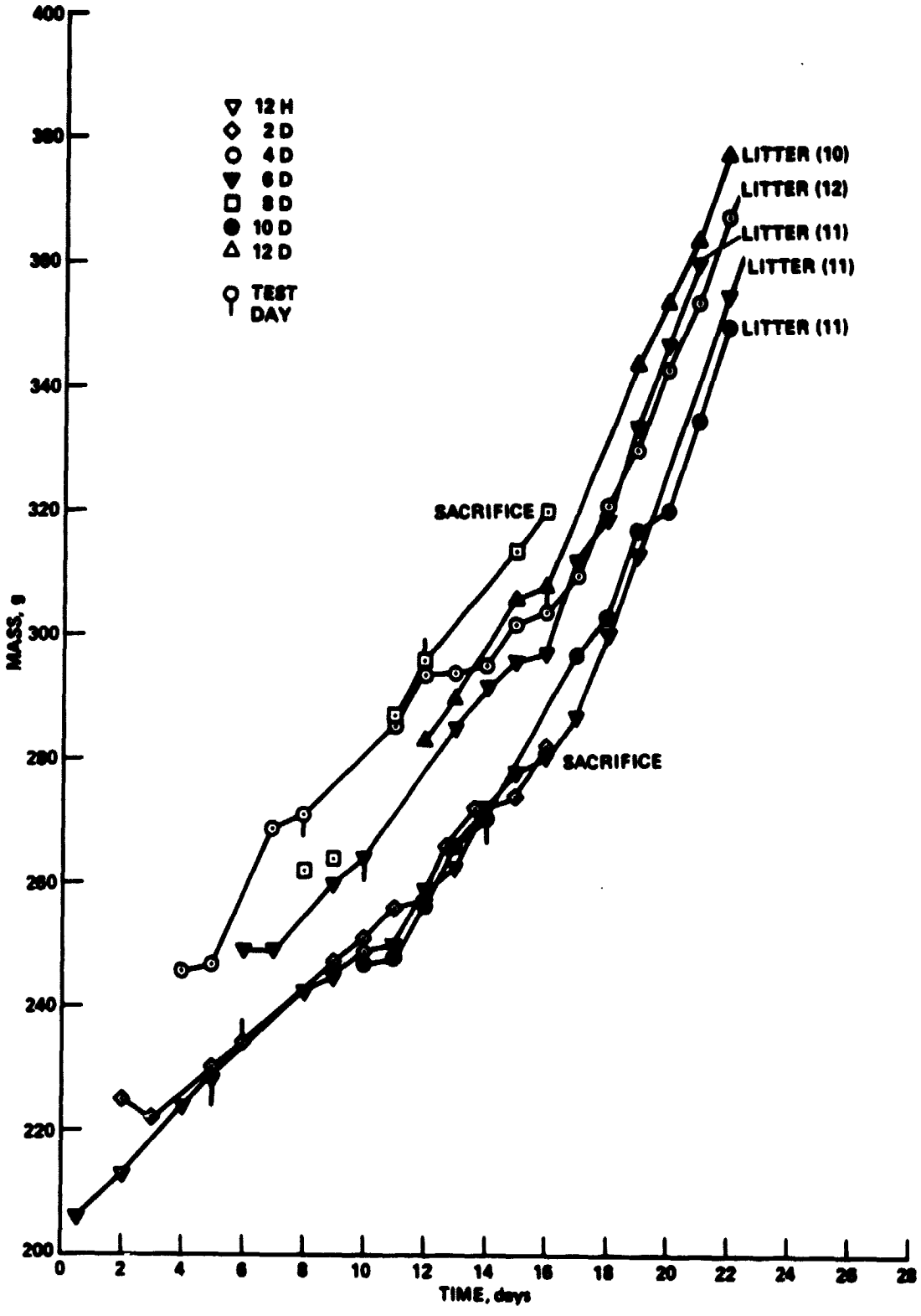
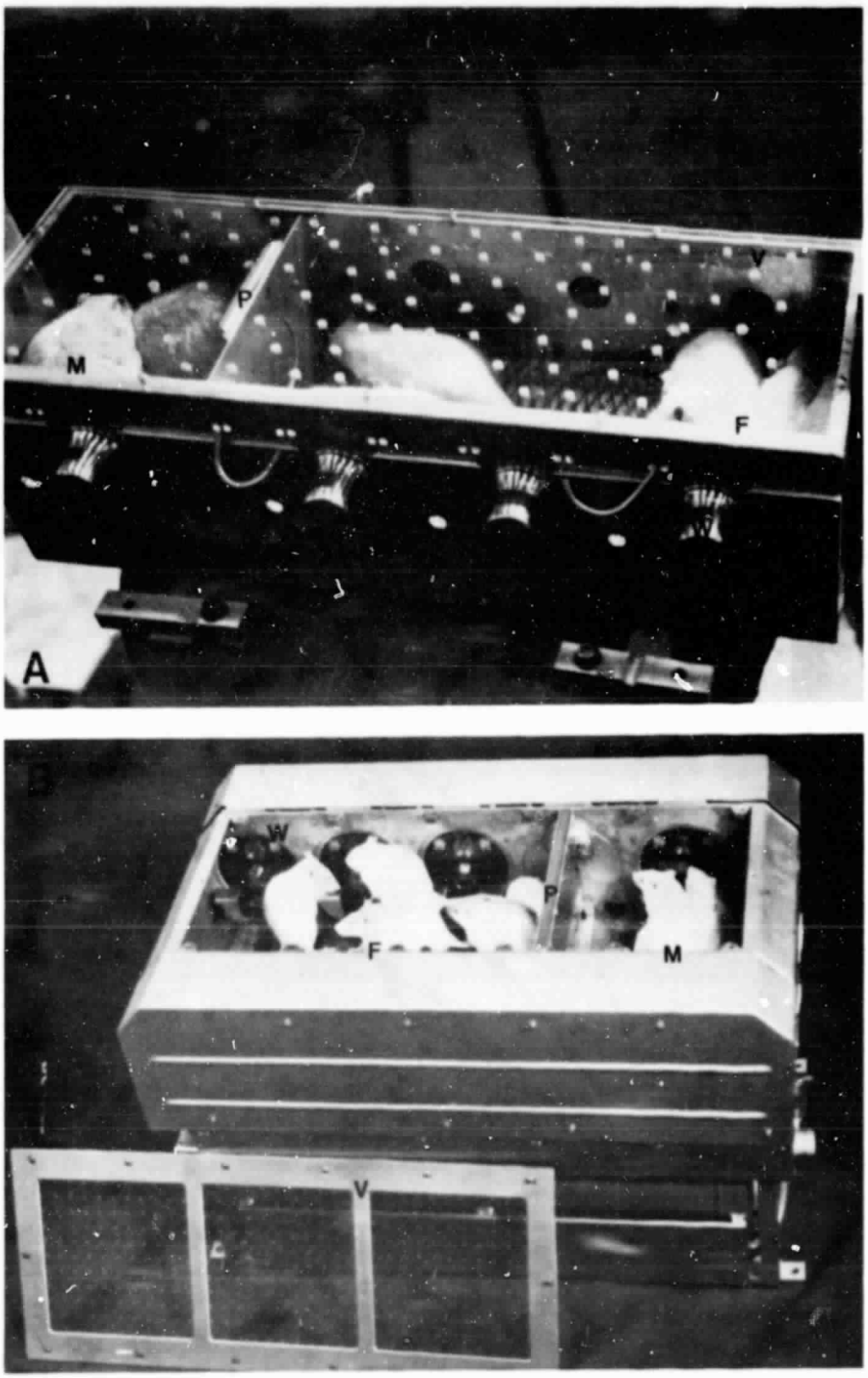


Figure 3.- Mass of pregnant control females as a function of gestation day: Group TA-3 (sacrificed with corresponding TA-2 female).



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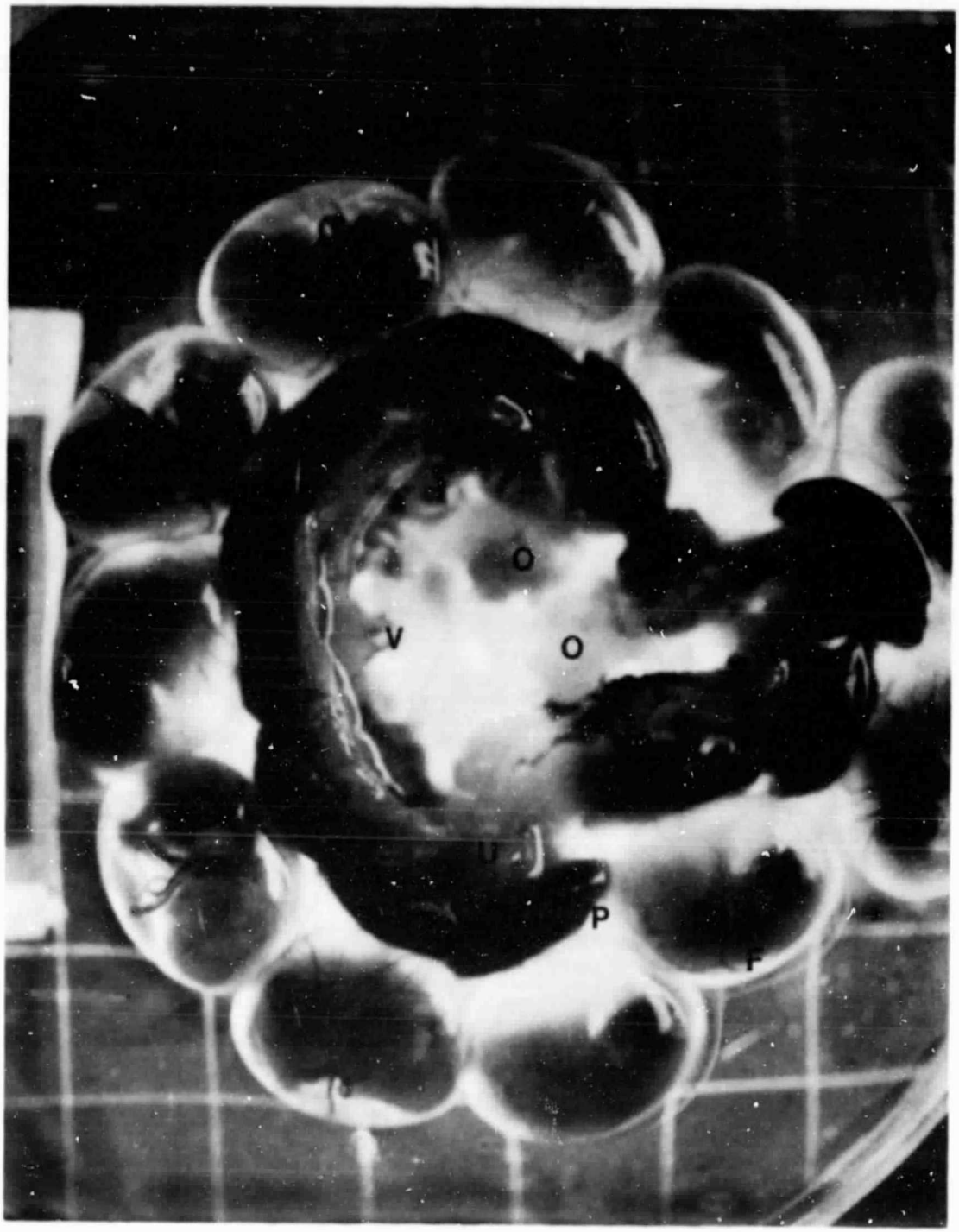

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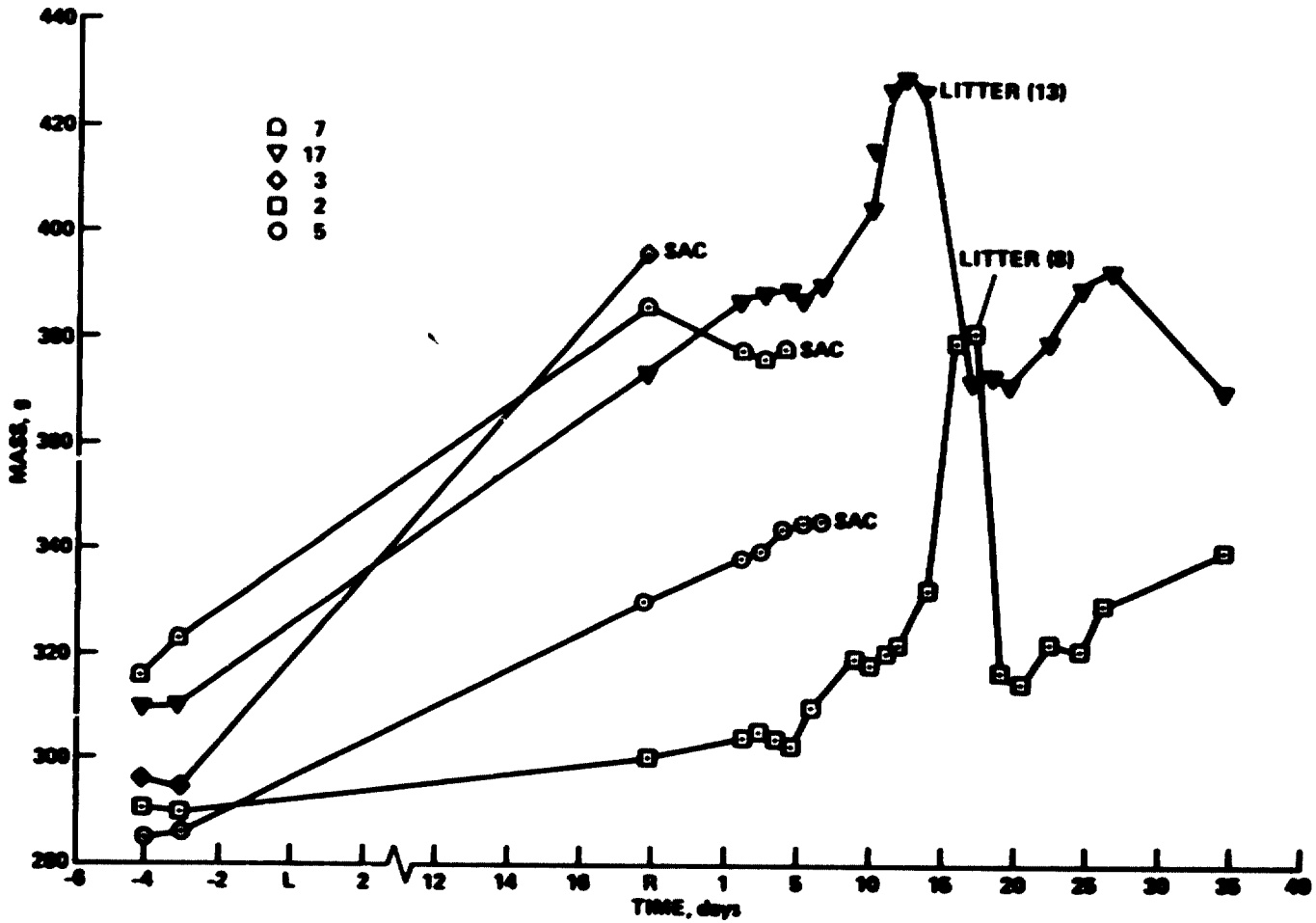


Figure 6.- Mass of females during full flight simulation: Group TS-1 (not handled during incubation).

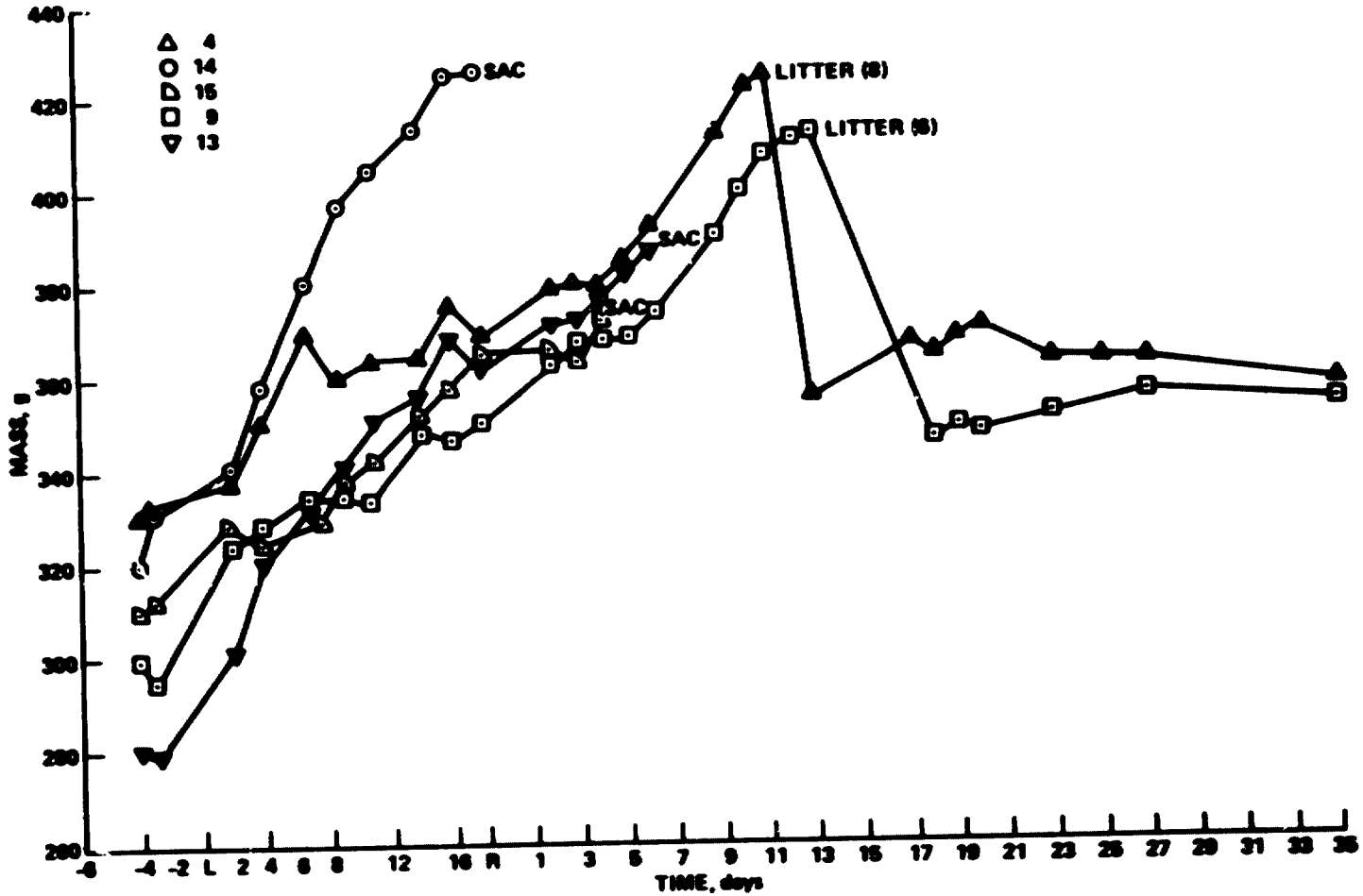


Figure 7.- Mass of females during full flight simulation: Group TS-2 (handled for weighing and vaginal smear).

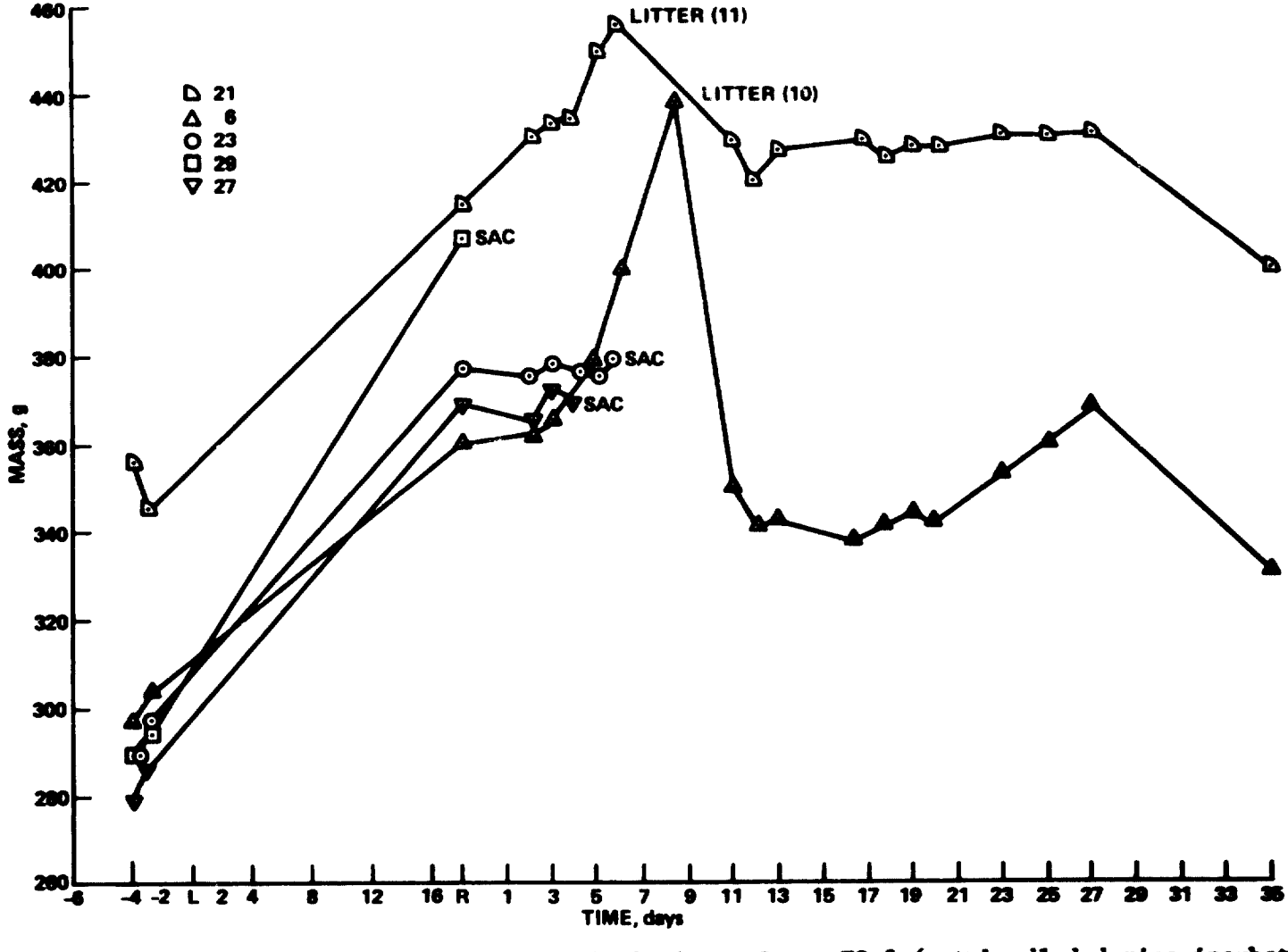
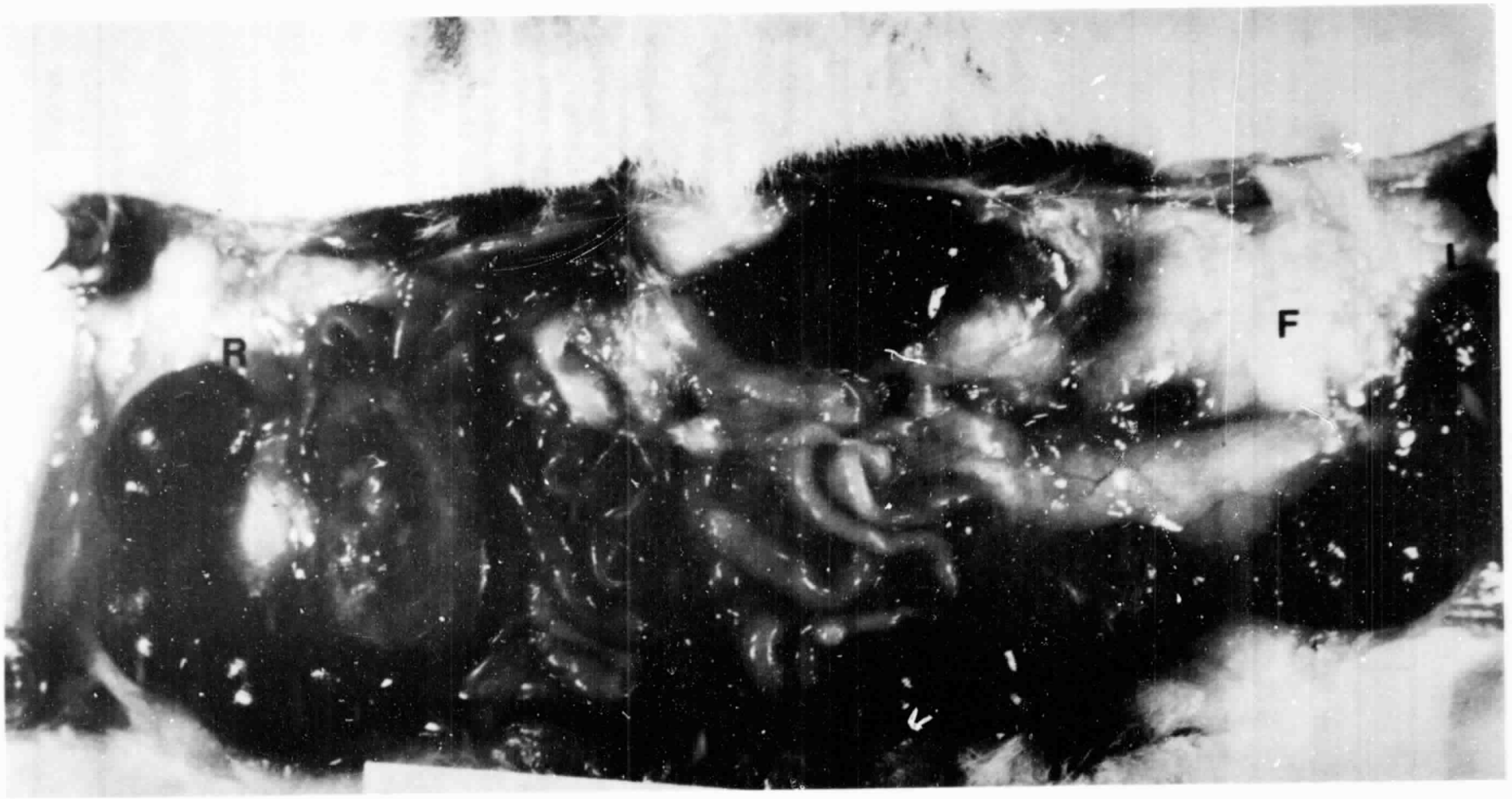


Figure 8.- Mass of females during full flight simulation: Group TS-3 (not handled during incubation).



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